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BIOSYNTHESIS OF STARCH

Abstract:

Evidence is presented which supports the proposal (Erlander, S.R., Enzymologia, 1958, 19, 273-283) that plant glycogen is a required intermediate in the synthesis of starch. Its synthesis temporarily ceases in a specific cell after a period of about three days, and at that point debranching enzymes are then activated which remove its exterior A-chains. The partially debranched glycogen is amylopectin and the removed branches are degraded by soluble starch synthases (SSS I and II) to produce ADPglucose (ADPGlu) which is the sole source of glucose for the production of amylose by the granular bound starch synthase (GBSS). Two independent cytosol/plastid transport systems activate either phosphorylase (from transported ADPGlu) or its back-up system SSS II (from transported Glu-6-P). Both use ADP glucose pyrophosphorylase in the synthesis of glycogen. The linear chains of debranched amylopectin have the narrow *Poisson* size distribution, whereas those of linear amylose have the broad size distribution of an A-B condensation polymer. Thus amylose can not be a precursor to amylopectin. Inner, short A-chains, located particularly on the 3rd and 5th tiers of the precursor glycogen, account for changes in the A/B chain ratio and for clusters.

Introduction

About forty years ago it was proposed [1] that starch is synthesized in the plant, not by first synthesizing amylose-type chains, but rather by first synthesizing a highly branched plant glycogen from which both amylose and amylopectin are produced. Thus it was proposed [1] in 1958 that there must exist in the plant a debranching enzyme which removes exterior A-chains from this precursor glycogen and then allows these removed chains to be converted into amylose. The partially debranched glycogen then becomes, with its elongated exterior chains, amylopectin. And the newly created and elongated exterior chains, which now have become about twice the length of the average distance between branch points, allows the amylopectin to retrograde (form double helices) and thus to form starch granules. As pointed out by Wursch and Gumy [2], any exterior amylopectin chain which is equal to or less than eleven glucose units long will not participate in a retrogradation process. Thus starch can not form unless this partial debranching occurs first.

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About eleven years after the proposal of this glycogen precursor mechanism, the predicted, essential debranching enzymes were discovered [3-5] in sweet corn. Thus as stated in 1984 by Pan and Nelson [5]: "We believe that Erlander's hypothesis that sugary maize is defective in debranching enzyme activity is correct, but not his conjecture that phytoglycogen is a normal intermediate in starch synthesis." However, if all of the debranching enzymes are removed by genetic manipulation, then according to the proposed mechanism, the only product which should be formed is the precursor glycogen *if* the glycogen is indeed a "normal intermediate." And this is exactly what Mouille, et.al., [6] discovered in 1996 when they genetically eliminated the debranching enzyme and consequently found only a 9% branched polysaccharide and no starch (no amylopectin *and* no amylose). Further support of the proposed glycogen precursor mechanism was given when it was shown [7] that plant glycogen exists, not only in sweet corn, but also exists in dent, waxy and *ae* corn. In addition, other experimental data, as given in reviews both recently [8-10] and previously [11], also support this mechanism.

Furthermore, it is now known that the linear chains of amylose are synthesized by the granular bound starch synthase (GBSS). As pointed out previously [10, 11], there exists a quantitative relationship between the amount of branches removed from the glycogen and the amount of amylose found in starch granules. In order to retain this relationship and prevent glucose obtained from these debranched chains from entering the general pool for further glycogen synthesis, it was proposed [11] in 1970 that these undegraded chains must be connected together by some unknown mechanism to form amylose. In the extended glycogen precursor mechanism [10], it is shown that this previous explanation is not necessary since this quantitative relationship can be maintained even though the removed branches are degraded to glucose if it is assumed that the synthesis of the glycogen precursor is halted after three or four days of synthesis in a *specific* cell and before this glycogen is converted into starch.

The role of phosphorylase in starch synthesis

Initially, phosphorylase plus branching enzymes were proposed [1] as those enzymes which produced the precursor glycogen. However, it has now been generally concluded by others that the soluble starch synthases (SSS I and SSS II) produce the linear chains from ADPglucose (ADPGlu) using ADPglucose pyrophosphorylase (ADPGlu pp) and glucose-1-phosphate (Glu-1-P). In contrast, mechanisms [10] presented here illustrate that phosphorylase is still valid as the primary enzyme for the production of linear chains in the precursor glycogen and incorporate ADPGlu pp, which, as pointed out by Preiss [12], is essential for the synthesis of starch.

It was discovered by Akazawa and his group [13] that a translocator exists for the transfer of ADPGlu from the cytosol into the plastid. Afterwards Mohlmann, et al. [14] and Chen, et al. [15] observed that ADPGlu pp exists in both the cytosol and the plas-

tid and thus Glu-1-P could be converted into ADPGlu in the cytosol. In addition, Mohlmann, et al. [14], showed that two independent transport systems are used for starch synthesis: one for ADPGlu and another for glucose-6-phosphate (Glu-6-P). Based on this and other information, it was concluded [10] that these two independent transport systems lead to two mechanisms for the synthesis of starch: 1) the transport of ADPGlu involves phosphorylase, considered here as the primary mechanism, and 2) the transport of Glu-6-P involves SSS II, considered here as the back-up system.

The validity of using phosphorylase for starch synthesis has been supported by the recent results of Duwenig, et.al. [16] as well as by other results [10]. In addition, the SSS II enzyme can produce a polymodal debranched amylopectin, whereas the SSS I enzyme does not [8-10]. Consequently, since native starch contains a polymodal-type amylopectin, then this observation rules out the use of SSS I in starch synthesis and thus allows the SSS II system to be the potential back-up for starch synthesis if the phosphorylase system fails.

The glycogen precursor mechanism

The production of either ADPGlu or Glu-6-P in the cytosol is initiated by sucrose synthase which converts sucrose into UDPGlu plus fructose. These two components can then be converted into either ADPGlu or Glu-6-P. Using cytosolic ADPGlu pp and the enzymatic systems of Schaffer and Petreikov [17], then UDPGlu and fructose are changed into ADPGlu in the cytosol as follows: UDPGlu→Glu-1-P (using UDPGlu pp) →ADPGlu (using ADPGlu pp); and fructose→fructose-6-P (using fructokinase) →Glu-6-P (using phosphoglucoisomerase) →Glu-1-P (using phosphoglucomutase) →ADPGlu (using ADPGlu pp). Also for the back-up system, the sucrose could be converted into Glu-6-P in the cytosol by use of the same enzyme-type systems. Either ADPGlu or Glu-6-P is then transported into the plastid.

Using the phosphorylase as the primary mechanism, it is proposed [10] that the transported ADPGlu is converted into glycogen in the plastid by the following mechanism:

$$\text{ADPGlu} + \text{PPi} \rightarrow \text{ATP} + \text{Glu-1-P} \quad \text{(using ADPGlu pp)} \qquad (1)$$

$$\text{Glu-1-P} + G_{n-1} \rightarrow P_i + G_n \text{ (glycogen) (using phosphorylase and branching enzymes)} \qquad (2)$$

In the back-up system, the Glu-1-P (from transported Glu-6-P) is used by reversing Eq.1:

$$\text{Glu-1-P} + \text{ATP} \rightarrow \text{PPi} + \text{ADPGlu} \quad \text{(using ADPGlu pp)} \qquad (3)$$

$$\text{ADPGlu} + G_{n-1} \rightarrow \text{ADP} + G_n \text{ (glycogen) (using SSS II and branching enzymes)} \qquad (4)$$

Thus both systems rely on the critical ADPGlu pp enzyme to produce the glycogen.

It should be noted that in order to transfer the ADPGlu from the cytosol to the

plastid, there is involved an exchange with AMP [14]. In addition, the transfer of Glu-6-P from the cytosol to the plastid involves a counter exchange with Pi. In the case of the ADPGlu transport mechanism the production of the AMP can occur from fat metabolism in the plastid:

$$ATP + RCO_2H + HS\text{-}CoA \rightarrow AMP + PPi + RCO\text{-}S\text{-}CoA + H_2O$$

The summary of the reaction would be: ATP → AMP + PPi. Thus this reaction could supply the needed AMP for the translocation of the ADPGlu and in addition the needed PPi for Eq. 1. Plus the production of protein in the cytosol with the use of transfer RNA produces the same result: ATP → AMP + PPi. Extra PPi is also generated in the cytosol in the conversion of fructose to ADPGlu. The PPi could thus be supplied by a counter transfer with Pi. That is, it is known that Pi is involved in a homo transfer with itself (Pi) or in a hetero transfer with Glu-6-P [14]. Hence, it is possible that this same translocator could also transfer PPi with a counter transfer of Pi. In addition, AMP could be produced with the use of plastid myokinase: ADP → ATP + AMP [14]. Thus the necessary ingredients for Eq. 1 can be produced.

Each transfer mechanism is independent and it appears that each suppresses the other. Hence, when ADPGlu transport is used to produce starch, it suppresses the use of the Glu-6-P transport mechanism and vice-versa. With respect to the Glu-6-P transport mechanism, Eq. 3 shows that ADPGlu is produced which in turn can suppress phosphorylase (Eq. 2) which is used by the ADPGlu transport system. In addition, the production of Pi in Eq. 2 for the ADPGlu could compete with the transfer of Glu-6-P from the cytosol from the homo transfer of Pi/Pi or the hetero transfer of PPi/Pi.

Mohlmann, et al. [14] were puzzled as to why ATP inhibits starch synthesis through the ADPGlu transport mechanism, whereas ATP enhances and is required for the Glu-6-P transport mechanism. This apparent contradiction can be explained by the use of the phosphorylase system (Eqs. 1, 2) since now ATP becomes a product (Eq. 1) rather than a substrate (Eq. 3). If excess ATP is added to the ADPGlu transport mechanism, then that excess ATP will suppress starch synthesis by partially reversing Eq. 1. The result adds further proof for the proposed mechanism.

It was concluded previously [18] from a study of radioactive data on starch synthesis, particularly from the data given by Whistler and Young [19], that the synthesis of the plant glycogen occurs over a period of three or four days. After the glycogen synthesis reaches a critical point (perhaps at a maximum concentration of glycogen for the plastid), then it was proposed [10] that the enzyme system ADPGlu pp, and consequently the synthesis of glycogen, is stopped. After the branching enzymes have further branched the external chains of the newly synthesized glycogen without the addition of more glucose units (the production of short, external "stub" branches), then – and only then – are the debranching enzymes released, which in turn removes the stub

branches plus the newly formed A-chains to produce amylopectin.

Thus it is proposed that the resulting glycogen, from either the phosphorylase or the SSS II system, is converted into amylopectin plus (in non-waxy plants) amylose as follows:

G_n (glycogen) $\rightarrow$ G_{n-x} (amylopectin) + G_x (removed branch) (using debranching enzymes) (5)

G_x (removed branch) + ADP $\rightarrow$ G_{x-1} + ADPGlu (using SSS I and SSS II) (6)

ADPGlu (from removed branches only) + G_a (amylose) $\rightarrow$ ADP+ G_{a+1} (using GBSS) (7)

Hence, the partially debranched precursor glycogen becomes amylopectin (Eq. 5) and the removed branches become amylose (Eq. 6 and 7). The proposal that the synthesis of the precursor glycogen is stopped before the release of the debranching enzymes ensures that the only glucose units used in the synthesis of amylose are those glucose units which come from the removed branches. In this manner, as proposed initially [1] in 1958, there is established a quantitative relationship between the yield of amylose and the extent of debranching of the precursor glycogen.

The three or four day period for glycogen synthesis based on radioactive studies

The proposed mechanism [10] can explain why the maximum in yield of glycogen and the midpoint in the increase in the yield of starch both occurred at 7 p.m. (1900) for the 26th day sweet corn [20]. In addition, the mechanism explains why the yield of plant glycogen from dent and waxy corn for the 7 p.m.(1900) sample was about twice the yield of the 9 a.m. (0900) morning sample [7]. That is, if all of the glycogen were converted into starch in a single day rather than the proposed three or four day period, then essentially all of the glycogen would have disappeared by the following morning and consequently the yield of glycogen for the 9 a.m. (0900) sample should have been near zero rather than about one-half of the evening sample. Indeed, the existence itself of plant glycogen in dent, waxy and even *ae* corn [7], as well as its maximum yield at the midpoint of starch synthesis [20], backs the validity of the glycogen precursor mechanism.

In addition, the initially high radioactivity of the amylose, as observed by Whistler and Young [19], also supports the three or four day synthesis since this length of time allows a mixture of mature and immature glycogen to exist together in the plant, but each in separate cells. It is concluded that when the radioactive sucrose was inserted into the plant by Whistler and Young [19], then the more randomly labeled, immature glycogen was retained, whereas the mature glycogen, with radioactivity in only its exterior chains, was converted into starch. And since the debranching enzymes are proposed to be inactive until the glycogen is mature, then it is the exterior radioactive A-chains of these mature glycogens which are the first to be removed by the debranching enzymes to produce in this case an initially high radioactivity in amylose.

But this high radioactivity in amylose then tapers off as uniformly labeled precursor glycogens begin to be converted into starch after three or four days.

The use of the SSS II back-up system

The questions arises: Are there any examples of a shift from the proposed primary phosphorylase system to the SSS II system? As pointed out by Mohlmann, et. al. [14], corn (maize) and normal barley use the ADPGlu transport system, whereas wheat uses the G-6-P transport system. Moreover, such a transition from the phosphorylase system to the SSS II system could have occurred in the antisense experiments of Sonnewald, et. al. [21], where antisense repression of the major leaf plastidic type L phosphorylase took place. The transition would explain the continual production of starch despite the destruction of this phosphorylase. Another possible example is the conversion of Bomi to *shx* barley. It was concluded [8-10] from an analysis of the data of Schulman, et. al. [22] on the genetic change from Bomi barley to *shx* barley, that this genetic change involves a transition from phosphorylase to the SSS II enzyme system. In other words, the removal of one of the three SSS I enzymes (but none of the SSS II enzymes) in this conversion could not possibly account for the dramatic 69% loss of starch. That is, the proposed back-up system using SSS II produces a polymodal debranched amylopectin, but SSS I enzyme does not [8-10] and thus the SSS I can not be a major contributor (or even contribute at all) to the synthesis of the precursor glycogen.

In connection with this possible conversion of the phosphorylase system in Bomi to the SSS II system in *shx* barley, the phosphorylase system produces ATP (Eq. 1) which is in essence a direct transportation of ATP to the plastid. The ATP also inactivates the Glu-6-P transport system (the SSS II system) [14]. This extra energy could in part account for the greater yield of starch in the Bomi barley. Moreover, Mohlmann, et. al [14] observed (their Table 2) that the rate of starch synthesis using transported ADPGlu is about six times faster (99.5/16.8) than that rate produced by using the transported Glu-6-P. According to the proposed equations, this dramatic difference in rates for the two transport systems could establish phosphorylase as the primary system and, in addition, could account for the observed [22] loss of about two-thirds of the starch yield in going from Bomi to *shx* barley.

The polymer size distributions of debranched amylopectin chains and amylose

It was concluded in 1988 [23] and more recently [8] that the polymodal size distribution of the completely debranched amylopectin can be correlated with individual polymers. It was then proposed that these individual polymers have their origin in the tier structures of the precursor glycogen and its resulting amylopectin. For example, if there are no branches attached in its original structure (an A-chain), then its degree of polymerization would be approximately $x_n = 10$. However, the external A-chains of the

amylopectin would have a value of $x_n = 20$ or so, since in the precursor glycogen this external A-chain was a B-chain with one branch attached to it (before stub branching had occurred). Thus theoretically, there should be no chains in the amylopectin structure which produce a polymer of the size $x_n = 10$ or so. Examination of the polymodal behavior of debranched amylopectin chains shows, however, that there exists two and possibly three such polymers, with perhaps degrees of polymerization of about $x_n = 10$ and 12. These two short polymers are considered to be hidden in the interior structure and are produced because of steric hindrance in the growing precursor glycogen. Shorter polymers, of perhaps $x_n = 3$ or 4, are shown to exist in polymodal patterns of Schulman, et.al. [22] for debranched amylopectins from Bomi and *shx* barley starches, and these shorter polymers can be ascribed to remnants of the proposed stub branching. A method was developed in 1988 [23] and presently [8] which allows the calculation of the fraction of the assumed polymers in the mixture of the polymodal debranched amylopectin chains. In the calculations, it is assumed that each polymer is distinct and has the *Poisson* size distribution, which agrees with the observation by Bailey and Whelan [24] in 1961 that phosphorylase produces a synthetic amylose which has a *Poisson* size distribution.

Later, in 1994, Ong, et al. [25] made similar calculations using the Gaussian curve for size distribution calculations and similar results were obtained. However, the Gaussian curve becomes much broader at the higher molecular weights and thus when applied to the structure of amylopectin would produce less branching in the amylopectin interior which contradicts the properties of the branching enzyme and the greater steric hindrance in the more exterior chains.

In contrast to the *Poisson* size distribution for the various distinct polymers of debranched amylopectin, it was observed [8, 23] that linear amylose has an extremely broad size distribution and behaves as an A-B type condensation polymer. This contrast in size distributions shows that amylose can not be the precursor to amylopectin since different enzyme systems must be involved in their production. In other words, the granular bound starch synthase (GBSS) and SSS I do not produce a polymodal-type amylopectin [10], and thus do not synthesize linear chains in the same manner as the polymodal-producing phosphorylase or SSS II. Hence, amylopectin can not be produced by first synthesizing a short, precursor amylose polymer. Consequently, based on these size distributions, the amylose precursor mechanism is erroneous.

The branched polymers of amylose can also be examined by correlating the properties of these polymers with size distributions for various theoretical polymers using Flory's equations [26] for linear A-B condensation polymers and our mathematical extension [27] to represent multiple branched A-R-B_{f-1} condensation polymers. These equations were applied [8] to the results of Everett and Foster [28] and Takeda, et al.

[29]. Takeda, et al. [29] separated individual polymers of amylose at 40°C (two branched structures and one linear structure). A comparison [8] of the theoretical model with the experimental results [29] showed that one of the branched polymers of amylose behaved as an A-R-B_2 type condensation polymer, the other branched polymer as a non-statistical A-R-B_2 type condensation polymer (a transition polymer), and the third polymer as a completely linear A-B type condensation polymer.

The location of "hidden" a-chains in the interior structure of amylopectin

Baba, et al. [30] showed that debranching of their resulting radioactive amylopectin produced short linear chains which were not radioactive. These short chains, which appeared to have an approximate degree of polymerization of $x_n = 10$, most likely were the above "hidden" chains [10]. Further proof of this is given by the fact that *beta* – amylolysis of the amylopectin showed that the radioactivity was in the longer exterior chains ($x_n = 20$) of the amylopectin [30]. Thus the shorter, non-radioactive chains were located in the interior part of the amylopectin. Furthermore, the narrow size distributions of their resulting chains (both the non-radioactive short and radioactive long chains) illustrated that these polymers had the *Poisson* size distribution.

The position of these hidden, inner A-chains was also determined by comparing one variety of debranched amylopectin with another. That is, if shorter A-chains replace much longer B-chains to a greater extent in one variety than in another, then the difference between the two varieties should show a decrease in the longer B-chains when there is an increase in the shorter, inner A-chains. Theoretical *Poisson* polymers were used to produce theoretical curves for debranched amylopectins from wheat, tapioca and barley. In these theoretical curves only one A-chain was used ($x_n = 10$). Because of the very narrow width of the *Poisson* size distribution curves, the theoretical curve had a deep "dip" between ten and twenty glucose unit chain lengths. Similar dips in the debranched amylopectin curves also occurred as well. The amount of the $x_n = 30$ polymer had the following sequence: wheat (4.2%) < tapioca (6.2%) < barley (13.8%), which correlated with the sequence for the amount of the intermediate polymer (as measured by the percentage increase in the experimental dip over that of the theoretical dip): wheat (21%) > tapioca (17%) > barley (10%). Thus the decrease in the $x_n = 30$ polymer is followed by an increase in the $x_n = 12$ polymer. Consequently, the longer inner chain ($x_n = 12$ or so) must be located primarily in the third tier from the exterior of the precursor glycogen, whereas the shorter ($x_n = 10$) inner chain must be located primarily in the more interior fifth tier.

A similar comparison was made between Bomi and *shx* barley, where as discussed above, it is considered that the Bomi barley uses phosphorylase and the *shx* barley uses SSS II in the synthesis of the linear chains of glycogen. A comparison

showed that the $x_n = 12$ was greater for the Bomi barley and the $x_n = 10$ polymer was greater for the *shx* barley. But the greater amount of the $x_n = 12$ polymer in the Bomi barley was associated with lesser values for the $x_n = 30$, 40 and 70 polymers. Likewise, the greater amount of the $x_n = 10$ polymer was associated with lesser amounts of the $x_n = 50$, 60 and 80 polymers. In other words, just as the $x_n = 12$ polymer displaced the $x_n = 30$ polymer in wheat, tapioca and barley, so also the increase in the $x_n = 12$ polymer can be associated with a partial replacement(a decrease) in the $x_n = 30$ polymer, as well as a partial replacement, that is, decrease, in the $x_n = 40$ and 70 polymers. In other words, the location of the $x_n = 12$ polymer is in the third, fourth and seventh tiers of the precursor glycogen and that of the $x_n = 10$ polymer is in its fifth, sixth and eighth tiers. The larger, inner or "hidden" polymer therefore appears in general to be in the exterior tiers of the glycogen and amylopectin structures.

The statistical model for amylopectin and its precursor glycogen

A comparison was made between amylopectins and a model based on all possible structures (a statistical model) which have three branch points (a total of 5 structures) which is comparable to those structures which have six branches (132 structures) and seven branches (429 structures) as studied previously [31]. It is seen that the change in the ratio of A/B chains in going from glycogen to amylopectin can be explained by using hidden A-chains. The Meyer model for the precursor glycogen, modified by placing short, inner A-chains in its structure, gave similar results.

These hidden A-chains can also explain the presence of clusters. The size of these clusters, as described by Zhu and Bertoft [32], is approximately that of the three branched structures, or possibly slightly larger. Furthermore, these structures are produced from random branching, as in the formation of a statistical structure, since as pointed out by Zhu and Bertoft [32] at least three different structures exist in these clusters. Hence, the statistical model, with inner, short A-chains and with a *Poisson* size distribution for the linear chains, represents the amylopectin and its clusters.

Conclusions

The proposed glycogen precursor mechanism [1, 10] for starch synthesis is supported by experimental evidence: the predicted debranching enzymes have been found and the predicted glycogen intermediate has also been found. That is, a 9% branched glycogen was observed by Mouille, et.al. [6], when the debranching enzymes were inactivated and 8% branched glycogens were isolated not only from sweet corn, but also from dent and waxy corn solubles, as well as the 6% branched glycogen from *ae* corn solubles [7]. The postulated use of phosphorylase as the primary enzyme for one of two predicted independent pathways for starch synthesis [10] is also supported by

experimental evidence and is based on the observed [14] existance of two transport systems. The continuation of starch synthesis when the phosphorylase is inactivated, possibly by destroying the translocator for ADPGlu, can be explained by a switch to a back-up system, that is, the transport of the Glu-6-P into the plastid. This in turn must instigate the production of the precursor glycogen with SSS II and branching enzymes. Both mechanisms (phosphorylase and SSS II) are dependent upon the use of the critical enzyme ADPGlu pp.

The statistical model and the proposed glycogen precursor mechanism illustrate that the amount of removed branches correlates with the amount of amylose found in starches, that "stub" branching is needed to produce the 8.0% branching observed [7] in dent, waxy and sweet corn glycogens, that the $x_n = 30$ polymer exists in amylopectins but is reduced in amount because of a partial, but variable, replacement of these chains with short A-chains ($x_n = 12$), and that slightly shorter A-chains ($x_n = 10$) have also replaced many of the $x_n = 50$ polymers in wheat and barley amylopectins. Moreover, amylose can not be a precursor to amylopectin since the linear amylose behaves as a broad A-B condensation polymer, whereas the debranched amylopectin chains behave as *Poisson*-type polymers with a very narrow size distribution.

Aggregation (retrogradation) of amylopectin occurs by the debranching of the exterior chains after glycogen synthesis stops. Further aggregation must occur with proteins to produce reversible aggregates in waxy (disaggregation in 0.05M sodium phosphate at pH = 7 [33] and irreversible aggregates in dent corn amylopectins [8].

Emes and Neuhaus [34] consider the possibility that the ADPGlu transport system allows the use of ADPGlu pp in the cytosol, the consequent transfer of the ADPGlu, and then the production of linear glucose chains by the direct use of the SSS enzymes without the use of ADPGlu in the plastid. However, even with activity of ADPGlu pp in the cytosol, there is still activity of this enzyme in the amyloplast. Consequently, it is concluded that the above proposed mechanism using phosphorylase explains the use of the ADPGlu pp in *both* the cytosol and the plastid.

One of the arguments against the proposed phosphorylase mechanism is that as seen in Eq. 2 there is a production of phosphate which it is argued would inhibit the ADPGlu pp enzyme. However, as pointed out by Tetlow, et.al. [35], phosphate does not accumulate in the plastid during starch synthesis even though it could, and that its removal can not at present be accounted for. Thus they consider that some unknown transport mechanism transfers the phosphate into the cytosol: "Presumably, mechanisms exist in the amyloplasts for the removal of the additional phosphate" [35]. Consequently, these data indicate that at present unknown transport mechanisms could also remove the phosphate generated by Eq. 2 (or PPi in Eq.3), such as a counter exchange of Pi and PPi.

Genetic alterations of the genes controlling the ADPGlu pp show that the activity of the ADPGlu pp can be increased tremendously [36]. With respect to this increase in ADPGlu pp activity, in unpublished work by M.J. Emes, as reported by J. Preiss [37], a mutation increased by four fold the activity of this enzyme (from 110 to 339 activity units) and at the same time substantially increased the yield of starch. Interestingly, with this increase in both the ADPGlu pp activity and the yield of starch, there was also a dramatic *decrease* in the activity of the soluble starch synthases (from 3527 to 2231 activity units) plus a slight increase in the activity of phosphorylase (from 355 to 376 activity units). The corresponding one-third drop in SSS activity indicates that the SSS is not used in this case in the production of the precursor glycogen, but most likely only for the conversion of the removed branches into amylose, as proposed in Eq. 6 above. On the other hand, the presence of phosphorylase plus simultaneous increases in *both* the activity of phosphorylase and that of ADPGlu pp illustrate that in this case phosphorylase in involved in starch synthesis. Furthermore, the use of phosphorylase in the ADPGlu transport system explains the mystery [14] of why added ATP suppresses the ADPGlu transport system (Eqs. 1 and 2) but in contrast enhances the Glu-6-P transport system (Eqs. 3 and 4). Moreover, the mutation results [37] may have involved a shift from the Glu-6-P transport system to the ADPGlu transport system and this shift could also have increased both the activity of ADPGlu pp (since *both* cytosolic and plastidic ADPGlu pp would be used) and the yield of starch (since the rate of starch synthesis is six times faster for the ADPGlu transport system [14].

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BIOSYNTEZA SKROBI

Streszczenie

Przedstawiono dowód podtrzymujący sugestię (Erlander Enzymologia 1958, 19, 273-283), że glikogen jest niezbędnym związkiem pośrednim w syntezie skrobi. Jego synteza w specyficznych komórkach czasowo ustaje po około trzech dniach i uaktywniają się enzymy odcinające rozgałęzienia usuwając jego zewnętrzne łańcuchy A. Glikogen z częściowo usuniętymi odgałęzieniami stanowi amylopektynę, a odcięte łańcuchy ulegają degradacji przez syntazę rozpuszczalnej skrobi (SSS I i II) dając ADP-glukozę (ADPGlu), która jest jedynym źródłem glukozy przekształcanej w amylozę przez syntazę gałeczkowej skrobi związanej (GBSS). Dwa niezależne układy transportu cytosol/plastyd albo aktywują fosforylazę (z transportowanej ADPGlu) lub wspierającego układu SSS (z transportowanej Glu-6-P). W syntezie glikogenu oba te systemy korzystają z fosforylazy ADPGlu. Liniowe łańcuchy amylopektyny pozbawione odgałęzień mają niewielki zakres rozrzutu rozmiarów wg *Poissona*, podczas gdy łańcuchy amylozy, polimeru z kondensacji A z B, mają bardzo zróżnicowane rozmiary. Zatem amyloza nie może być prekrsorem amylopektyny. Wewnętrzne, krótkie łańcuchy A znajdujące się przede wszystkim na 3 i 5 jednostkach glukozowych glikogenu odpowiadają za zamiany stosunku liczby łańcuchów A i B (A/B) i za powstawanie klasterów.